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THE NATURE OF HEPATIC AND SPLENIC SYMPATHIN

BY

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Because of the desire to identify the substances liberated by adrenergic nerves, much work has been devoted to the search for really sensitive and specific methods of detecting and estimating adrenaline and allied substances in blood. Some of the difficulties of these estimations in heparinized blood from cats and man were discussed by Gaddum, Peart, and Vogt (1949), who selected five sensitive tests for detailed study. By making parallel quantitative assays, closely allied sympathomimetic amines can be distinguished from one another. Using these criteria, Peart (1949) found that all the evidence supported the view that the active material released by the adrenergic nerves in the cat's spleen was *nor*adrenaline, although smaller amounts of adrenaline were sometimes also liberated. Great similarity in the effects of hepatic nerve stimulation and intraportal injections of *nor*adrenaline have been recorded (for references see West, 1950), and now we have estimated the concentrations of the two amines in the plasma after stimulation of the splenic and hepatic nerves, utilizing the formula developed by Bülbring (1949).

METHODS

Collection of hepatic blood.—Cats anaesthetized with chloralose were used. The hepatic nerve was dissected free from the artery and divided centrally. The incision down the midline just below the diaphragm was extended laterally just below the course of the lowest rib and parallel to it on the right side of the cat. When the muscles were retracted and the liver pushed gently aside, easy access to the right hepatic vein was gained. A loop of thread was fixed in position round the inferior vena cava between the liver and the adrenal glands, and another similarly above the diaphragm, artificial respiration being applied. When these threads were extended, a little pocket of blood formed at the opening of the hepatic veins into the vena cava. Blood samples of about 5 ml. were withdrawn every thirty seconds from the hepatic vein by means of a 5 ml. syringe fitted with a No. 15 needle (D'Silva, 1936). When the needle was withdrawn from the vein, there was no haemorrhage, and subsequent samples were obtained by inserting the needle into the vein through the original wound. Heparin was injected intravenously and the blood samples were transferred from the syringe to graduated centrifuge tubes standing in ice. The plasma was separated immediately by centrifugation. In about half the experiments, the adrenals were excluded from the circulation by ligatures. Stimulation of the nerve was through platinum electrodes with an ordinary coil (Faradic stimulation at 7.5 cm. on 4 V.), and usually lasted for one minute. During this time, the two threads round the inferior vena cava were extended and two blood samples were

obtained. A third sample was usually secured after stimulation had ceased, and the plasma from the three samples was bulked. The control plasma was obtained usually by bulking samples secured before, and 10 minutes after, stimulation, the two threads being extended as described above.

Collection of the splenic blood.—Cats anaesthetized with chloralose were used. The splenic nerve was dissected free from the artery and divided centrally. In most cases, vascular connexion of the spleen with the stomach and greater omentum was divided between ligatures. Splenic venous blood was led from a cannula in the splenic vein through a rubber tube and small reservoir to the femoral vein. When blood samples were required, the reservoir was replaced by a graduated centrifuge tube standing in ice. Heparin was used as the anticoagulant, and plasma samples were obtained by rapid centrifugation. In about half the experiments, the adrenals were excluded from the circulation by ligatures. Stimulation of the nerve was through platinum electrodes with an ordinary coil (Faradic stimulation at 7.5 cm. on 4 V.) and usually lasted for one minute. During this time and for the next two minutes, the volume of blood collected was usually 3–6 ml. The control plasma was obtained by bulking samples secured before, and 10 minutes after, stimulation.

Assay methods.—Samples of the plasma were used in three pharmacological tests: (1) An isolated uterus from a non-pregnant rat in dioestrus, sensitive to 10^{-10} adrenaline or 5×10^{-9} *nor*adrenaline in a bath of Tyrode solution at 37° C. (2) An isolated rectum of a week-old chick in a similar bath, sensitive to 5×10^{-10} adrenaline or 10^{-8} *nor*adrenaline. (3) The chronically denervated nictitating membrane of a cat under chloralose, recorded isotonically, and contracted by 10^{-8} adrenaline or *nor*-adrenaline if given into the sidearm of a special T-shaped cannula in the carotid artery (Gaddum, Peart, and Vogt, 1949). Solutions of *l*-adrenaline and *l-nor*adrenaline were prepared in 0.01 N-HCl.

When mixtures of adrenaline and *nor*adrenaline were added to control samples of plasma, accurate estimates were obtained by these tests when the activities were calculated by the formula employed by Bülbring (1949). As little as 4 per cent of one of the amines could be accurately measured in a mixture of the two.

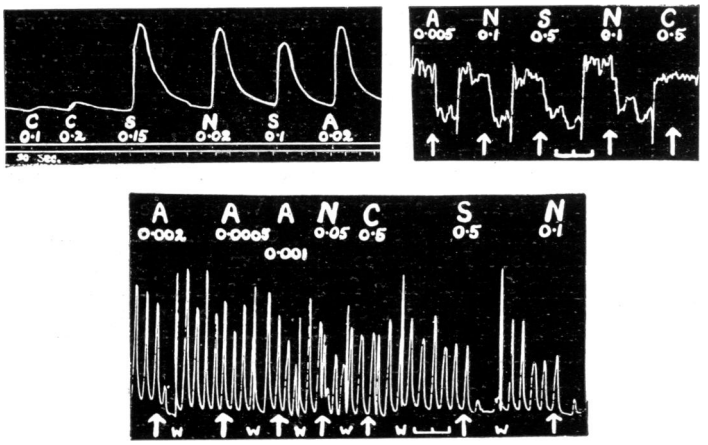

FIG. 1.—Parallel assays (Exp. 5). Splenic plasma before (C) and after (S) stimulation of nerves. A, adrenaline; N, *nor*adrenaline. Doses in μg. and ml. Records: top left, nictitating membrane of cat; top right, chick rectum; bottom, rat non-pregnant uterus in dioestrus. *Nor*-adrenaline equivalent is 0.2 μg. *nor*adrenaline per ml. plasma. Time in 30 sec.

TABLE I

CONCENTRATION OF HEPATIC SYMPATHIN IN THE POST-STIMULUS SAMPLE OF PLASMA IN TERMS OF ADRENALINE AND *nor*ADRENALINE (μG./ML.)

Sympathin estimated as	Nictitating membrane of cat	Chick rectum	Rat uterus in dioestrus
l-adrenaline (A)	0.75	0.025	0.01
*l-nor*adrenaline (N) ..	0.75	0.50	0.50
Ratio A/N	1.0	0.05	0.02

Calculated value (Bülbring, 1949) gives mean of 0.66 μg. *l-nor*adrenaline per ml. Adrenaline value is zero. Value on rat uterus in oestrus (uncalculated) = 0.50 μg. *l-no* adrenaline per ml.

RESULTS

Parallel quantitative assays by different methods were carried out on the plasma obtained in each experiment (Fig. 1). The estimates were taken in pairs and values for adrenaline and *nor*adrenaline calculated by Bülbring's formula. An example of hepatic sympathin is shown in Table I, where the calculated value for adrenaline is zero—i.e., all the sympathin was present as *nor*adrenaline. This was confirmed by assaying the sample on the isolated uterus of the non-pregnant rat in full oestrus (i.e., when cornified cells are in the vagina smear). Both *nor*adrenaline and adrenaline stimulate this tissue (Mann, 1949), and the estimate in terms of *nor*adrenaline agreed well with the calculated value. This stimulation was abolished by dibenamine. Further evidence that the main active material is *nor*adrenaline is provided by the fact that both the "post-stimulus" samples and *nor*adrenaline stimulated the non-pregnant isolated rat uterus in early oestrus (when nucleated epithelial and cornified cells are in the vaginal smear), whereas adrenaline relaxed this tissue at this stage of the oestrous cycle (Mann, 1949); see Fig. 2.

In a series of 13 experiments (Table II), estimates of the hepatic sympathin gave a mean value of 0.48 μg. *l-nor*adrenaline per ml. plasma. This value agrees with that found by West (1950) on whole blood samples (0.4 μg. *l-nor*adrenaline per ml.). Gener-

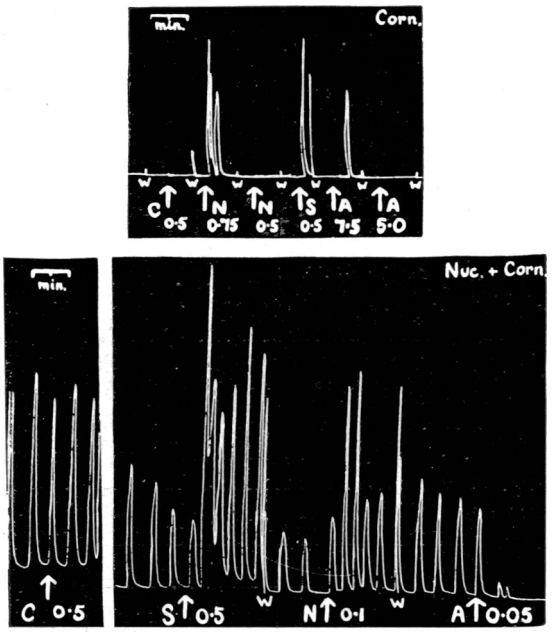

FIG. 2.—Confirmatory evidence (Exp. 5). Splenic plasma before (C) and after (S) stimulation of nerves. A, adrenaline; N, *nor*adrenaline. Doses in μg. and ml. Records: top, non-pregnant rat uterus in oestrus (only cornified cells in vaginal smear); bottom, same in early oestrus (epithelial nucleated and cornified cells in vaginal smear).

TABLE II

CONCENTRATION OF HEPATIC SYMPATHIN IN POST-STIMULUS SAMPLES OF PLASMA

Exp.	μg./ml. plasma		Exp.	μg./ml. plasma	
	*l-nor*adrenaline	*l*-adrenaline		*l-nor*adrenaline	*l*-adrenaline
1	0.11	0	8	0.05	0.002
2	0.33	0	9	0.17	0
3*	1.05	0	10*	1.05	0
4	0.16	0	11	0.32	0
5*	1.00	0	12	0.25	0.003
6*	1.05	0	13	0.66	0
7	0.08	0			

* Adrenal vessels open in these exps.; tied in all other exps.

ally higher values were obtained when the adrenal vessels were open, but traces of adrenaline were found by calculation in two samples when these vessels were closed. A few of the assays were complicated by the presence of unknown interfering substances in the plasma, but these were reduced to a minimum by cooling the samples immediately after collection, centrifuging, and testing the plasma on the same day as it was collected. There was little doubt from the calculations that the main active material was *nor*adrenaline and not adrenaline.

TABLE III

CONCENTRATION OF SPLENIC SYMPATHIN IN THE POST-STIMULUS SAMPLE OF PLASMA IN TERMS OF ADRENALINE AND *no*-ADRENALINE (μG./ML.)

Sympathin estimated as	Nictitating membrane of cat	Chick rectum	Rat uterus in dioestrus
l-adrenaline (A)	0.25	0.01	0.0025
*l-nor*adrenaline (N) ..	0.25	0.20	0.125
Ratio A/N	1.0	0.05	0.02

Calculated value gives mean of 0.25 μg. *l-nor*adrenaline per ml. Adrenaline value is zero. Value on rat uterus in oestrus (uncalculated) = 0.20 μg. *l-nor*adrenaline per ml.

TABLE IV

CONCENTRATION OF SPLENIC SYMPATHIN IN POST-STIMULUS SAMPLES OF PLASMA

Exp.	μg./ml. plasma		Exp.	μg./ml. plasma	
	*l-nor*adrenaline	*l*-adrenaline		*l-nor*adrenaline	*l*-adrenaline
1	0.10	0.001	9*	0.05	0.010
2	0.10	0	10*	0.05	0.002
3*	0.05	0	11*	0.05	0.002
4*	0.20	0	12*	0.05	0.002
5	0.20	0	13	0.17	0.001
6	0.11	0	14	0.18	0
7	0.11	0	15	0.25	0
8*	0.30	0			

* Adrenal vessels open in these exps.; tied in all other exps.

An example of splenic sympathin calculations is shown in Table III, where the adrenaline value is again zero. When assayed on the rat uterus in full oestrus, the estimate in terms of *nor*adrenaline agreed well with the calculated value. These " post-stimulus " samples of splenic sympathin also stimulated the rat uterus in early oestrus. In a series of 15 experiments (Table IV), estimates of the splenic sympathin gave a mean value of 0.13 μg. *l-nor*adrenaline per ml. plasma. This value also agrees with that found by West (1950) on whole blood samples (0.25 μg. *l-nor*adrenaline per ml.). Traces of adrenaline were found by calculation in six of the plasma samples. Ligation of the adrenal vessels did not affect the results. In two further experiments, no sympathomimetic activity was detected despite sensitive test objects. Usually the sympathin content of plasma collected after a second stimulation of the nerves later in the same experiment was lower than that in the first sample.

Discussion

Most of the previous work on hepatic and splenic sympathin has clearly indicated that the active material is not adrenaline and is probably *nor*adrenaline. The work reported here, based on direct assay methods, has proved that this is so. In both sympathins, only traces of adrenaline have been detected, but this evidence supports the views of Bacq and Fischer (1947), who obtained evidence of such a mixture in extracts of the splenic nerves of the horse and cow, and of Euler (1948), who estimated sympathomimetic activity in extracts of various organs including the liver. According to Blaschko (1942) *nor*adrenaline is the probable precursor of adrenaline in the body, so it is to be expected that mixtures of the two amines will be found in the blood as a result of sympathetic activity under varying conditions. It is hoped to identify the sympathin liberated by other adrenergic nerves by means of the technique described here.

Summary

1. Stimulation of the hepatic and splenic nerves in cats caused the appearance in the plasma of an active substance which produced contraction of the nictitating membrane of the cat, relaxation of the isolated rectum of the chick and of the isolated uterus of the non-pregnant rat in dioestrus, contraction of the isolated uterus of the non-pregnant rat in oestrus and in early oestrus, when nucleated epithelial and cornified cells are present in the vaginal smear.

2. Parallel quantitative assays on the plasma showed that the main active substance was *nor*adrenaline ; this was present in the blood of the hepatic veins in a mean concentration of 0.48 μg./ml. (13 experiments), and in the blood of the splenic vein in a mean concentration of 0.13 μg./ml. (15 experiments).

3. Smaller amounts of adrenaline were sometimes also liberated.

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